EXHIBIT 12

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Lack of repellency of three commercial ultrasonic devices to the German cockroach (Blattodea: Blattellidae)

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Abstract Three commercial ultrasonic devices (A, B, and C) were tested for their ability to repel the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae), in Plexiglas® enclosures. Device A generated peak frequencies at 26 kHz and 34 kHz, and produced a 95 ± 1 dB sound pressure level (SPL) at 50 cm distance (0 dB = 20 $\log_{10}$[20 μPa/ 20 μPa]). Device B generated peak frequencies at 27 kHz and 35 kHz, and produced a 92 ± 4 dB SPL. Device C generated a wide range of frequencies between 28–42 kHz and produced an 88 ± 2 dB SPL. Ultrasound from any of the three devices did not demonstrate sufficient repelling ability against the German cockroach in the tests. The result failed to provide evidence that ultrasonic technology could be used as an effective pest management tool to repel or eliminate the German cockroach.

Key words *Blattella germanica*, ultrasonic devices, urban pest management
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Introduction

The German cockroach, *Blattella germanica* (L.), is a serious household and public health pest worldwide (Brenner, 1995). Numerous ultrasonic pest-control devices are manufactured and sold in many countries, including the US (Anonymous, 2005). Manufacturers claim that these ultrasonic devices are able to control or repel many types of pests including the German cockroach. Since the ultrasonic pest-control devices are not regulated under the US Federal Insecticide, Fungicide and Rodenticide Act, the US Environmental Protect Agency does not require efficacy data for these devices as it does for chemical pesticides. Scientific data on the performance of these devices are seriously lacking (Anonymous, 2005). A few tests of ultrasonic devices in repelling the cockroach were conducted in the early 1980s (Ballard & Gold, 1983; Ballard *et al*., 1984; Gold *et al*., 1984; Schreck *et al*., 1984; Koehler *et al*., 1986). Data reported by Ballard *et al*. (1984) suggested that ultrasound was able to repel the cockroach, while results from other tests were negative. Customer responses are also controversial. Some claim that the devices are very effective but others declare they do not work at all (Anonymous, 2005). In 2001, the US Federal Trade Commission sent warning letters to > 60 manufacturers and retailers of ultrasonic pest-control devices, stating that efficacy claims about these devices must be supported by scientific evidence (US Federal Trade Commission, 2001). A manufacturer of ultrasonic devices in the US recently approached us to test three of their commercial models against a wide variety of insects, including the German cockroach. This paper describes the sound patterns produced by the three devices, and quantifies their efficacy in repelling the cockroach under laboratory conditions.

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Materials and methods

Insects

A German cockroach colony was obtained from the

Department of Entomology, Kansas State University, Manhattan, Kansas, US. Insect cultures were reared with Pedigree® dog food (Kal Kan Foods, Vernon, California, US) in 3.3-liter plastic cereal storage containers with lids (Servin' Saver™, Rubbermaid Home Products, Wooster, Ohio, US). The insect colony had never been exposed to ultrasound. A plastic cup (6.5 cm diameter × 6.0 cm high) containing cotton saturated with distilled water was placed in each container to provide water for the insect culture. Cultures were held in the laboratory at 24–27℃ and 75%–80% RH.

Ultrasonic devices and sound output measurements

Three commercial ultrasonic devices, labeled as A, B, and C for proprietary reasons, were evaluated in Plexiglas® enclosures. Sound measurements were recorded from 11 units for each of devices A and B, and 14 units of device C. For each device, sound measurements recorded included peak frequencies produced, sound cycles, and sound pressure levels (SPLs). Measurements were made using a Brüel and Kjær (B & K) type 4939 condenser microphone, B&K type 2670 preamplifier, and B&K NEXUS conditioning amplifier (Brüel & Kjær, Norcross, Georgia, US). Data were collected using a Tektronix 544A digitizing oscilloscope. Measurements were calibrated using a B&K type 4231 sound level calibrator (Brüel & Kjær, Norcross, Georgia, US). Measurements were made at a distance of 50 cm from the unit's transducer.

Test procedures

Paired Plexiglas® enclosures, each measuring 1.2 m × 1.2 m × 1.2 m, were constructed (Fig.1) and placed in a room in an east-west orientation. The test room was an empty storage room before it was used for the study. The room had no windows and was quiet. The paired enclosures were connected at the bottom front corner by a 91 cm-long square conduit (7.5 cm × 7.5 cm) made of cardboard. Plexiglas® gates were placed at the junctions of the conduit, such that enclosures could be opened or closed, and when opened, allowed insects to freely move between the enclosures. A 6.5 cm-diameter hole was drilled at the center in the top surface of each enclosure. An ultrasonic unit was mounted outside the hole and the space between the ultrasonic unit and the Plexiglas® of the enclosure was sealed with glue to prevent insects escaping. The speakers for the units were fitted within the holes and faced directly toward the centers of the enclosure's floors (Fig. 1). In the control tests, ultrasonic units were replaced with plastic covers. Sound measurements within an enclosure were made at the center of each of 16 quadrates, 0.03 m × 0.03 m per quadrate, on the floors of the enclosures for each device.

Tests with each ultrasonic device and the control were replicated four times using a paired design in a random order. For each ultrasonic device, different ultrasonic units were used in each replication. Prior to insect releases, two 9-cm Petri dishes, one containing 20 g of Pedigree® dog food and the another containing cotton balls saturated with distilled water, were placed at the center of the floor in each enclosure. In each paired test, 100 nymphs and adults of mixed ages and sexes (80% nymphs and 20% adults) held in two 0.45-liter glass jars (50 insects per jar) were released at the center of the floor of each enclosure and allowed to

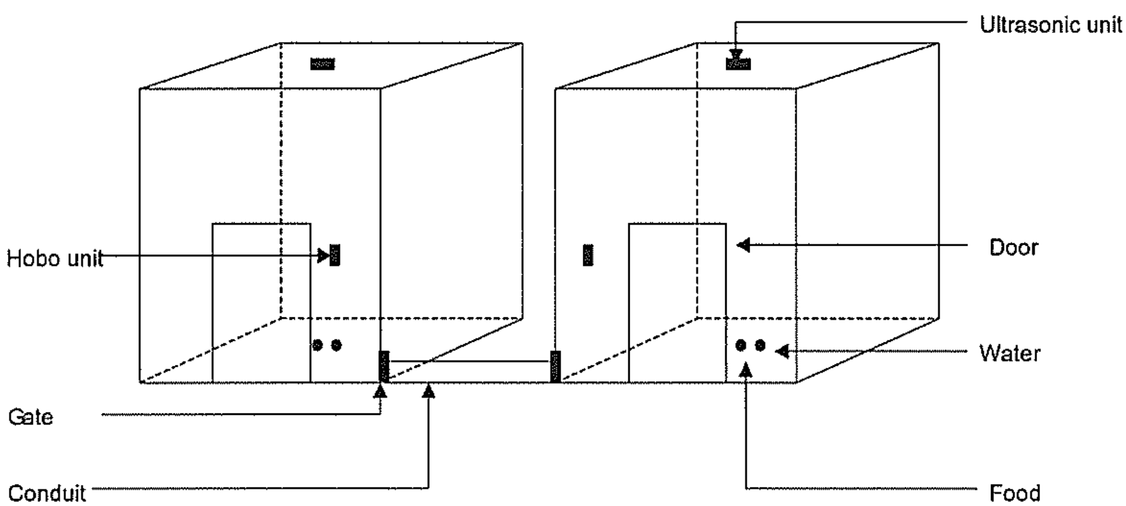

Fig. 1 Diagram of paired Plexiglas® enclosures, each measuring 1.2 m × 1.2 m × 1.2 m, connected at the bottom front corner by a 91 cm-long square conduit (7.5 cm × 7.5 cm) made of cardboard. Plexiglas® gates placed at the junctions of the conduit and enclosures could be opened or closed, and when opened, allowed insects to freely move between the enclosures.

acclimatize for 24 h. The Plexiglas® gates at the junctions were closed during this time period and then were opened to allow insects to freely move between the enclosures for another 24 h (day 1). After the 48-h acclimatization period, one ultrasonic unit in one of the enclosures was turned on (active) for 6 days (days 2–7) and the ultrasonic unit in the other enclosure remained off (inactive) at the same time. In the control tests, there were no ultrasonic units on either enclosure. This 7-day test constituted a single replication. The number of cockroaches in each enclosure was visually counted daily between 10:00–11:00 am and the number of dead cockroaches in the enclosures was also recorded at the end of each test. Enclosures were covered with black plastic sheets to exclude light and the covers were removed only briefly to facilitate counting. The Plexiglas® gates at the junctions were closed before the plastic covers were removed for insect-counting and reopened after the enclosures were recovered with the plastic sheets.

Microprocessor-based sensors (HOBO units, Onset Computer Corporation, Pocasset, MA, US) mounted in each enclosure were used to record temperatures and humidity levels. The temperature and relative humidity during all tests was 22.5–25.6℃ and 47.5%–82.0%, respectively. However, a majority of the temperatures ranged between 23℃ and 24℃ and the relative humidity was between 65% and 80%.

Data analysis

To determine the distribution of the SPLs within an enclosure, contour maps were generated using Surfer Surface Mapping System (Golden Software, Golden, Colorado, US) (Keckler, 1995) based on the means of SPLs recorded at each of the 16 quadrates in the bottom surface of the enclosure for each device. Data on the number of dead cockroaches in the enclosures found at the end of each test and the daily number of cockroaches in the paired enclosures were analyzed by paired *t*-tests, SAS PROC TTEST procedure (SAS Institute, 1996), to determine differences between device status (active and inactive) for each of the three devices at a specific date and between the enclosures located in the west and east sides in the control tests.

Results

Sound outputs

Ultrasonic device A generated peak frequencies at 26 kHz and 34 kHz (Fig. 2A). The device produced a 95 ± 1 dB SPL at 50 cm (0 dB=20 $\log_{10}$[20 μPa/20 μPa]). Similarly, device B generated peak frequencies at 27 kHz and 35 kHz (Fig. 2B), and produced a 92 ± 4 dB SPL. Device C units generated a wide range of frequencies from 27.7 to 42 kHz (Fig. 2C). The device produced an 88 ± 2 dB SPL.

The sound waveform plots showed that device A had a 0.123 s sound cycle (Fig. 3A). In each sound cycle, there were two pulse groups with eight pulses in each group. The interval between the two pulse groups was 0.038 s. In one group, a pair of weaker pulses was followed by a pair of

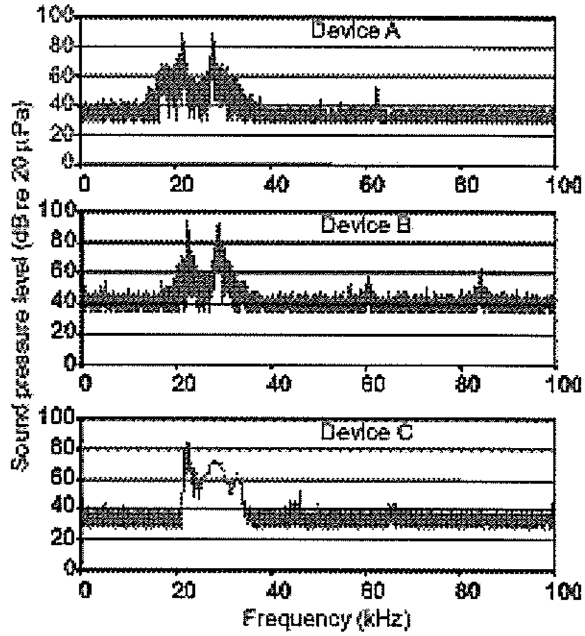

Fig. 2 Sound frequency spectrums generated from three commercial ultrasonic devices at 50 cm distance.

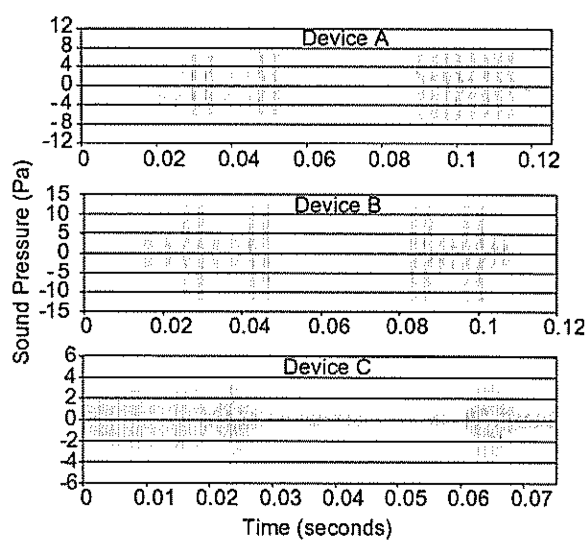

Fig. 3 Sound waveform graphs generated from three commercial ultrasonic devices at 50 cm distance.

stronger pulses. Device B also had the same duration (0.123 s) as device A for one sound cycle (Fig. 3B). This device also generated two groups of pulses similar to device A with eight pulses in each group. However, unlike device A, in a pulse group, a pair of weaker pulses were followed by a pair of stronger pulses, and in another pulse group a pair of stronger pulses were followed by a pair of weaker pulses. Device C had a single sound cycle that was 0.075 s (Fig. 2C). This device generated three groups of pulses, and each pulse group was characterized by multiple pulses.

The SPL in the enclosure without ultrasound when the other enclosure had an active ultrasonic unit was negligible (below the level of 0.01 Pa). The SPLs from devices A, B, and C at the bottom level within an enclosure with an active ultrasonic unit ranged from 83.7–91.6, 82.7–88.9, and 82. 3–88.1 dB, respectively (Fig. 4). The SPLs at the central area of the floors were somewhat higher than those recorded from the border area close to the side walls; but, in general, the SPL distribution at the bottom surface in an enclosure was relatively uniform and similar among the three devices.

German cockroach responses

The cockroaches survived well during the 7-day test period across all the tests, with an average of 4.8–7.3 cockroaches dying during this period in each enclosure (Table 1). In addition, there were no significant differences in the number of dead cockroaches ($t < 1.57$, $P > 0.2152$) between the enclosures with active and inactive ultrasonic units for all three devices and between the enclosures located in the west and east sides in the control tests, indicating ultrasound from any of the three devices did not cause any significant mortality.

In the control tests, the number of cockroaches in the enclosures located in the east and west sides was similar and was not significantly different through the 7-day test period except on the third day, which was significantly different ($t = 3.67$, $P = 0.034\,8$). However, the difference on the third day was small, (only 3 insects) compared to an average of 67.8 insects per enclosure. The remaining cockroaches that were unaccounted for (not visible) were in the conduits connecting the enclosures. In addition, in the tests with ultrasonic devices, the number of cockroaches observed on the first day before the ultrasonic units were turned on was also not significantly different between the paired enclosures ($t < 0.71$, $P > 0.527\,5$) across all the three devices (Table 1). These results suggest that the insects were evenly distributed between the paired enclosures in the control tests and in the tests with ultrasonic devices before the ultrasonic units were turned on.

In the paired tests with ultrasonic devices after one unit was turned on (active), the number of cockroaches in the enclosures with active ultrasonic units was consistently lower than that found in the enclosures with inactive units for all the three devices and throughout the 6-day period (days 2–7) (Table 1). The differences ranged 12–27 insects per enclosure for device A, 27–50 insects for device B, and 16–26 insects for device C, which represent approximately 9%–19%, 20%–35%, 13%–21% of total cockroaches observed in the paired enclosures, respectively. However, the paired t-tests indicate that these differences were not statistically significant ($P > 0.05$) for all three devices and throughout the 6 days except for device B on day 6, which was significant ($t = 3.73$, $P = 0.033\,6$). These results indicate that ultrasound produced from these devices did not repel cockroaches in the test conditions.

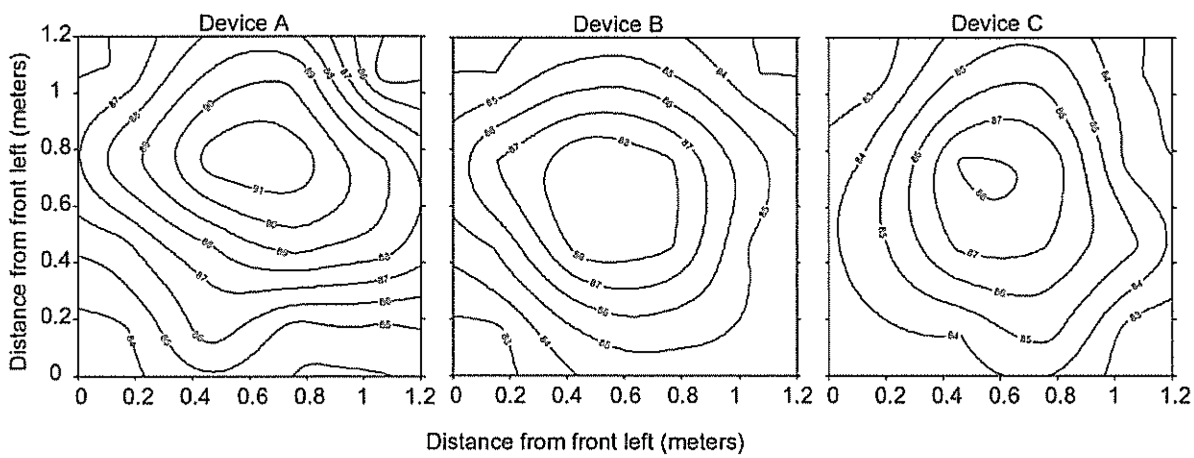

Fig. 4 Sound pressure levels (dB, 0 dB = 20 $\log_{10}$[20 μPa/20 μPa]) at the bottom level within an enclosure.

Table 1 Number (mean ± SE)† of the German cockroach in choice enclosures in absence or presence of ultrasound.

Device	Status	No. of dead insects 7 days after insect introduction	Daily no. of insects in the enclosures: Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	West	5.8 ± 1.9 a	80.8 ± 6.3 a	74.0 ± 5.0 a	69.3 ± 3.4 a	67.3 ± 4.2 a	64.5 ± 1.8 a	63.5 ± 2.3 a	61.0 ± 0.8 a
	East	7.3 ± 1.3 a	80.0 ± 7.7 a	71.5 ± 7.9 a	66.3 ± 3.3 b	69.5 ± 5.4 a	65.5 ± 2.3 a	64.0 ± 1.8 a	64.5 ± 3.6 a
A	Inactive	5.0 ± 2.7 a	68.3 ± 13.7 a	82.0 ± 7.2 a	76.7 ± 11.9 a	73.8 ± 13.9 a	76.0 ± 14.6 a	75.5 ± 15.0 a	78.3 ± 15.4 a
	Active	4.8 ± 2.0 a	77.0 ± 11.9 a	55.3 ± 13.9 a	64.3 ± 16.9 a	60.0 ± 13.0 a	61.8 ± 13.5 a	62.0 ± 12.0 a	61.8 ± 9.0 a
B	Inactive	5.0 ± 2.1 a	78.5 ± 2.5 a	86.0 ± 10.7 a	87.8 ± 8.9 a	95.0 ± 11.3 a	87.5 ± 8.2 a	85.3 ± 5.6 a	85.0 ± 7.8 a
	Active	5.5 ± 2.7 a	74.3 ± 3.1 a	52.0 ± 4.8 a	49.3 ± 7.9 a	45.3 ± 6.1 a	53.8 ± 8.2 a	47.5 ± 5.2 b	47.8 ± 5.9 a
C	Inactive	6.3 ± 2.7 a	64.0 ± 11.6 a	72.5 ± 14.1 a	69.7 ± 14.3 a	70.8 ± 12.6 a	76.3 ± 10.9 a	76.7 ± 8.8 a	82.3 ± 10.6 a
	Active	5.8 ± 1.8 a	76.0 ± 11.3 a	51.8 ± 4.1 a	53.7 ± 7.4 a	53.5 ± 4.5 a	50.3 ± 6.3 a	52.8 ± 7.1 a	57.3 ± 8.1 a

†Means in the paired enclosures at a specific observation date followed by different letters are significantly different ($P < 0.05$; paired t-test).

Discussion

All of the three devices apparently produced very strong ultrasounds as the manufacturers claimed, but data from our tests did not provide evidence that these devices can repel or eliminate the German cockroach as the manufacturers claimed. The insect colony used in the study had never been exposed to ultrasound. Therefore, it should be a good insect source for the study and the results generated from this colony should reflect the general insect populations. In field and laboratory conditions, we also found that ultrasound produced from these devices was unable to repel three ant species (Huang *et al.*, 2002). Most of the previous tests also showed ultrasonic devices were ineffective in repelling or eliminating the German cockroach (Ballard & Gold, 1983; Gold *et al.*, 1984; Schreck *et al.*, 1984; Koehler *et al.*, 1986). The only positive finding was that of Ballard *et al.* (1984). They reported that sound emitted from a commercial ultrasonic device could repel the German cockroach in the laboratory. The biological basis and importance of the positive results are difficult to interpret. The different results observed by Ballard *et al.* (1984) might be due to the differences in the sound properties produced from the device tested. The ultrasonic device they evaluated swept through frequencies of 30–65 kHz from 1.8–4.0 times per second. The units produced a 60–69 dB SPL at a 2 m distance. The test environmental conditions and insect colony might also contribute in part to the observed results. However, the repellency of ultrasound against the German cockroach reported by Ballard *et al.* (1984) was partial and was only significant at the α = 10% level. Therefore, results from Ballard *et al.* (1984) also indicate that the device could not repel the German cockroach as sufficiently as an effective pest management tool.

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Notes

This paper reports research results only. Mention of a proprietary product name does not constitute an endorsement for its use by Louisiana State University AgCenter or Kansas State University.

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